

ABSTRACT OF THE DISCLOSURE

5       Methods and materials are provided for stably  
introducing any gene into a specific locus in the genome of a  
microorganism such as yeast without the addition of any drug  
resistance genes. Specifically provided herein are new  
genetically engineered inositol-overproducing Saccharomyces  
10   cerevisiae strains obtained by using a novel set of yeast  
integration plasmids that allow the safe, stable, and controlled  
introduction of homologous as well as heterologous genes into  
the host genome. In particular, specific loci of the S.  
15   cerevisiae yeast genome can be targeted with single or multiple  
copies of a specific gene that is desired to be expressed or a  
given set of specific genes that the host can use without the  
addition of any drug resistance genes. The principles of this  
new methodology can also be used for the construction of other  
recombinant yeast and bacterial strains as well as higher  
eukaryotic cells.